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Short communication

In vitro study on the interaction of mechanism of tricyclic compounds with bovine serum albumin

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Abstract

The mechanism of interaction of five phenothiazine drugs with bovine serum albumin has been investigated using fluorescence spectroscopy, circular dichroism and equilibrium dialysis methods. It was found that the phenothiazine ring common to all drugs makes major contribution to interaction. However, the nature of alkylamino group at position 10 influences the protein binding significantly. Binding affinities could be related to parachor values of drugs. Stern–Volmer plots indicated the presence of static component in the quenching mechanism. Results also showed that both tryptophan residues of protein are accessible to drug molecules. The high magnitude of rate constant of quenching indicated that the process of energy transfer occurs by intermolecular interaction forces and thus drug-binding site is in close proximity to tryptophan residues of BSA. Binding studies in presence of hydrophobic probe, 8-anilino-1-naphthalein-sulphonic acid showed that there is hydrophobic interaction between drugs and the probe and they do not share common sites in BSA. Fluorescence intensity data in the presence of additives showed that hydrophobic interactions play a significant role. Small decrease in critical micellar concentration of anionic surfactant, sodium dodecyl sulfate in the presence of drugs showed that the ionic character of drugs also contribute to binding. Thermodynamic parameters obtained from data at different temperatures showed that the binding of phenothiazine drugs to BSA involve hydrophobic bonds predominantly. The CD spectrum of BSA in presence of drugs shows that binding of drugs leads to change in the helicity of the protein. The binding of these drugs to BSA based on dialysis experiment has been characterized by association constant (K) and the number of binding sites (n).

Keywords: Tricyclic compouds; Binding sites; Bovine serum albumin; Interaction studies

1. Introduction

The interaction of protein with various ligands create a basis of an interlocked set of dynamic processes providing a communication and regulation pathway within and between different structures of a living organism. Drug–protein interactions are important since most of the administered drugs are extensively and reversibly bound to serum albumin and drug is transported mainly as a complex with protein. The nature and magnitude of drug–protein interaction significantly influences the biological activity of the drug

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[1–3]. The binding parameters are useful in studying the pharmacological response of drugs and design of dosage forms [4,5]. Serum albumin being the major binding protein for the drugs and other physiological substances is considered as a model for studying drug–protein interaction in vitro [6].

Phenothiazines, an important class of tricyclic compounds, are widely used as anticholinergic, antihistaminic and antipsychotic drugs. Some aspects of binding of a few tricyclic psychoactive drugs to plasma proteins have been reported using gel filtration and micro calorimetric techniques [7–10]. These techniques are laborious and time consuming and the results, at times, are not reproducible. Moreover, the nature of binding forces is not clear. In vitro binding of phenothiazine to human erythrocytes [7] and alpha acid glyco protein [9,11] has been reported. However, there is no general agreement about the relative contribution of hydrophobic and ionic interaction and also which part of the molecule that is phenothiazine ring or substituents at positions 2 or 10 on the phenothiazine ring are involved in binding. Moreover, very few studies involving fluorescence technique are available [12] to understand the drug–protein interaction. Fluorescent probe, 8-anilino-1-naphthalein-sulphonic acid (ANS), which can be used as a valuable tool to understand the nature of interaction involved [1,13] is not employed by many researchers. In view of this, we planned to carry out the detailed investigations on the interaction of phenothiazines with BSA using fluorescence spectroscopy, circular dichroism and equilibrium dialysis. This is the first attempt wherein three analytical techniques are employed to investigate the mode of interaction of selected phenothiazines with BSA.

2. Experimental

2.1. Reagents and materials

Serum albumin bovine (BSA, Fraction V, approximately 99%; protease free and essentially γ-globulin free) was obtained from Sigma Chemical Company, St Louis, USA. Phenothiazine derivatives were obtained as gift samples from different manufacturers. All other materials were of analytical reagent grade. The solutions of drugs and BSA were prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. BSA solution was prepared based on molecular weight of 65,000.

2.2. Apparatus

Fluorescence measurements were performed on a Hitachi spectrofluorometer Model F-2000 equipped with a 150 W Xenon lamp and slit width of 10 nm. A 1.00 cm quartz cell was used for these studies.

CD measurements were made on a JASCO-810 spectropolarimeter using a 1.00 cm cell at 0.2 nm intervals, with three scans averaged for each CD spectrum in the range of 200–300 nm.

2.3. General procedure

2.3.1. Phenothiazine-BSA interaction

Some preliminary studies were carried out to select optimum protein and drug concentrations for drug–protein interaction. On the basis of preliminary experiments, BSA concentration was kept fixed at 10 μM and drug concentration was varied from 5 to 120 μM. Fluorescence spectra were recorded at room temperature (29 °C) in the range 310–500 nm upon excitation at 296 nm in each case. The absorbances of drug–protein mixtures in the concentration range employed for the experiment did not exceed 0.05 at the excitation wavelength in order to avoid inner filter effect.

2.3.2. Thermodynamics of drug–protein interactions

Thermodynamic parameters for the binding of drugs to BSA were determined by carrying out the binding studies at three different temperatures, 13, 29 and 35 °C by spectrofluorimetric method. Other conditions being same as described previously in Section 2.

2.3.3. Binding studies in the presence of hydrophobic probe

Experiments were also carried out in the presence of hydrophobic probe, ANS. In the first set of experiments, the interaction of drug and ANS with BSA was studied under identical conditions. BSA concentration was kept fixed at 10 μM and drug/ANS concentration was varied (4–25 μM). Fluorescence spectra were recorded in the range of 310–500 nm upon excitation at 296 nm. In the second set of experiments,

BSA–ANS interaction was studied in the presence and absence of 5, 10, 15, and 20 μM drugs. BSA concentration was again kept fixed at 10 μM and ANS concentration was varied from 4 to 25 μM. Fluorescence spectra were recorded in the range 390–550 nm upon excitation at 370 nm.

2.3.4. Critical micellar concentration (CMC)

CMC of sodium dodecyl sulphate (SDS) was determined in the presence and absence of 40 μM drugs using hydrophobic probe, ANS [14]. ANS concentration was kept fixed (60 μM) and SDS concentration was varied from 1.5 to 9.0 mM. Fluorescence spectra were recorded in the range 390–500 nm upon excitation at 370 nm.

2.3.5. Effects of additives

The fluorescence spectra of albumin-phenothiazines were recorded in presence and absence of various additives viz., urea, dextrose, magnesium stearate, starch and saturated lauric acid at 344 nm after excitation at 296 nm. The concentration of BSA and drug was fixed at 20 and 40 μM, respectively, and that of each additive was maintained at 20 μM.

2.3.6. Effect of paracetamol on drug–protein interactions

Protein–drug binding was studied in presence and absence of paracetamol using fluorescence spectroscopy. Emission spectra were recorded in the range of 310–500 nm.

2.3.7. Surface tension measurements

Surface tension of phenothiazine drug solution (each of 0.75% separately) prepared in phosphate buffer of pH 7.4 containing 0.15 M NaCl solution at 29 °C was determined by drop weight and drop number methods using a stalagmometer.

2.3.8. Circular dichroism (CD) measurements

A stock solution of 0.1 μM BSA was prepared in 0.01 M phosphate buffer containing 0.15 M NaCl solution. The BSA to drug concentration was varied (1:1, 1:3 and 1:5) and the CD spectrum was recorded.

2.3.9. Equilibrium dialysis method

Sample solution (10 ml) containing BSA (1.0%) and one of the phenothiazine drugs (TPM, LMM, EPH, PPP or PPC) in the concentration range 1.0–6.0 × 10^{-5} M was taken in 50 ml centrifuge tube and incubated at 37 °C for 6 h in a shaking water bath incubator. To each of the four dialysis tubings, 5 ml of the above reaction mixture was pipetted out. After closing, the dialysis tubing was immersed in standard phosphate buffer solution taken in a 100 ml bottle. The dialysis tubings were agitated up and down mechanically (6 h, 26 °C). At the end of dialysis experiment, the free phenothiazine drug from the buffer medium was extracted with chloroform. After evaporating the organic layer, the phenothiazine concentration was measured colorimetrically using 2 ml of 10 M phosphoric acid containing 0.0005 M Ce (IV) solution.

3. Result and discussion

The structures of phenothiazine derivatives employed in the present study are shown in Table 1.

3.1. Fluorescence studies

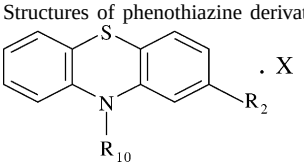
Fluorescence spectra of BSA were recorded in the presence of increasing amounts of various drugs. The spectra of one of the representative drugs, PPP are shown (Fig. 1). It was observed that the interaction of phenothiazine drugs with serum albumin did not result in any noticeable change in λ_{max} of tryptophan fluorescence in albumin. However, all the drugs were observed to quench the fluorescence of albumin. The fraction of drug bound, θ, was determined according to Weber and Young [15], and Maruyama et al. [16] using the equation,

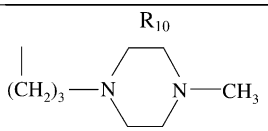
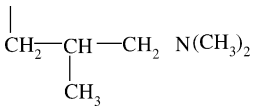
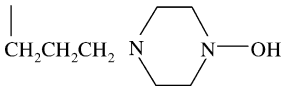
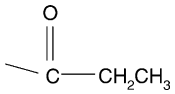
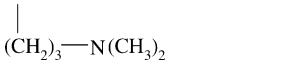
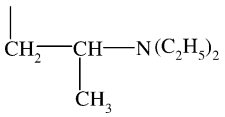
$$\theta = F_o - \frac{F}{F_o}, \quad (1)$$

where F and F_o denote the fluorescence intensities of protein in a solution with a given concentration of drug and without drug, respectively. The θ represents the fraction of site on the protein occupied by drug molecule. Fluorescence data was analyzed using the method described by Ward [17]. It has been shown that for equivalent and independent binding sites

$$\frac{1}{(1-\theta)K} = \frac{[Dt]}{\theta} - n[P_T] \quad (2)$$

Table 1
Structures of phenothiazine derivatives



Name of drug	R_2	R_{10}	X
Thioproperazine mesylate (TPM)	$-SO_2N(CH_3)_2$		2-CH_3SO_3H
Levomepromezine monomaleate (LMM)	–OMe		$C_4H_4O_4$
Propericiazine (PPC)	–CN		–
Propionylpromazine phosphate (PPP)			HCl
Ethopropazine hydrochloride (EPH)	–Cl		HCl

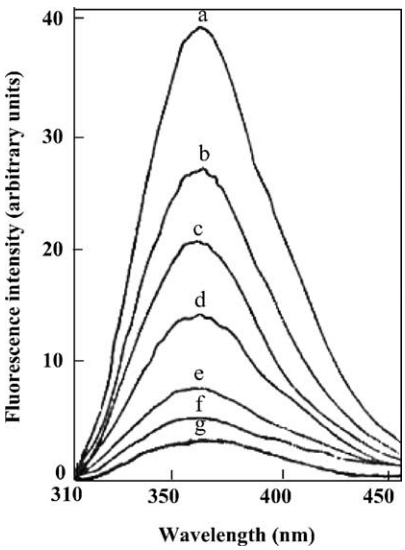


Fig. 1. Fluorescence spectra of BSA in the presence of PPP. BSA concentration was kept fixed (10 μM). PPP concentration was (a) 0 μM, (b) 20 μM, (c) 30 μM, (d) 40 μM, (e) 60 μM, (f) 80 μM and (g) 100 μM.

where, K is the association constant for drug–protein interaction, n the number of binding sites, [Dt] is the total drug concentration and $[P_T]$ is the total protein concentration. The plot of $1/1 - \theta$ versus $[Dt]/\theta$ for all the phenothiazines was recorded and the values of K and n were evaluated from the slope and intercept (Table 2). The K values were of the order of 10^4 and the number of binding sites varied from 1.0 to 4.5. Since the data fits Eq. (2) in all cases, it may be concluded that under the conditions of the experiment, all the binding sites are equivalent and independent. The order of K value is consistent with non-covalent interactions [8]. These values are supported by standard free energy change (ΔG_o) values obtained from the relationship, $\Delta G_o = -2.303RT \log K$ and is seen to be close to $-27\,\mathrm{KJ\,mol^{-1}}$ (Table 2). This shows that the phenothiazine ring common to all drugs makes major contribution to interaction. Phenothiazine ring is the primary hydrophobic portion of the molecule but substituents at positions 2 and 10 of the ring may have an effect on surface activity of the molecule.

Table 2
Binding parameters for the interaction of various phenothizine derivatives with bovine serum albumin

Phenothiazine	Association constant (K) ($\times 10^{-4}\,M^{-1}$)	Number of binding sites (n)	Standard free energy change ΔG_o (KJ mole^{-1})	Parachor ((N m^{-1})$^{1/4}$ m^3)
PPP	3.93	2.22	−26.57	679.8
PPC	3.84	4.57	−26.51	615.0
LMM	5.81	1.44	−27.55	918.8
EPH	4.12	1.46	−26.68	705.6
TPM	6.42	2.41	−27.80	1161.1

The binding is not directly related to hydrophobicity or hydrophilicity of substituents at position 2 on the ring. However, it plays an indirect role by affecting the intramolecular interaction in the molecule. The nature of alkyl amino group at position 10 appears to influence the protein binding significantly. For different drug samples, the K values vary in the order TPM > LMM > EPH > PPP > PPC. TPM has the longest alkyl amino chain and hence, has highest association constant followed by LMM. EPH, PPP and PPC which have slightly different chain length and also differ in substituents at 2 position of the phenothizine ring. A three-atom chain length is necessary to bring the protonated amino nitrogen into proximity with the substituent at 2. Isaacson [18] has emphasized that for optimum biological activity; there should be a critical size of substituents on the nitrogen of amino group. This indicates the importance of this part of the molecule for receptor attachment. Once the size requirement is met, the added chain length increases receptor-binding forces.

Parachor, which is a measure of molar volume of drug, was calculated for each drug from the atomic parachors and other structural features [19]. Values for different samples varied in the order TPM > LMM > EPH > PPP > PPC. The order of parachor values is in close agreement with the order of K values. It thus appears that molecular size of drug also plays a significant role in the binding of phenothiazine derivatives to serum albumin. The large size drug molecule probably has larger hydrophobic area, which can interact with hydrophobic surface on the protein molecule.

3.2. Stern–Volmer analysis

Fluorescence intensity data was also analyzed according to Stern–Volmer law [19,20],

$$\frac{F_o}{F} = 1 + K_q[Q] \quad (3)$$

by plotting F_o/F versus $[Q]$, where F_o and F are the steady state fluorescence intensities at 344 nm in the absence and presence of quencher (drug), respectively, and $[Q]$ is the total drug concentration. The Stern–Volmer plots (Fig. 2) showed positive deviation from straight line, suggesting the presence of a static component in the quenching mechanism [21]. A modified form of Stern–Volmer equation [21] that describes quenching data when both dynamic and static quenching are operative is

$$\frac{F_o}{F} = 1 + K_q[Q]\exp V[Q], \quad (4)$$

where, K_q is the collisional quenching constant or Stern–Volmer quenching constant and V is the static quenching constant. The value of V was obtained from Eq. (4) by plotting $[F_o/F\exp(V[Q])\}] - 1$ versus $[Q]$ for varying V until a linear plot was obtained. The

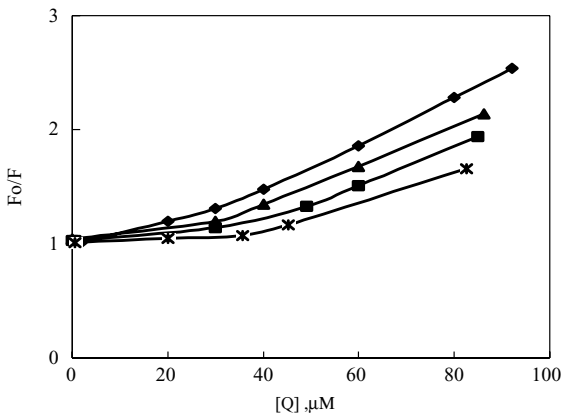


Fig. 2. Stern–Volmer plots for the binding of TPM (■) LMM (▲), EPH (◆) and PPC (✱) with BSA.

Table 3
Static and collisional quenching constants for various drug–protein systems

Phenothiazine	Static quenching constant (V) ($\times 10^{-3}\,M^{-1}$)	Collisional quenching constant (k_q) ($\times 10^{-4}\,M^{-1}$)	Collisional rate constant for quenching (k_q) ($\times 10^{-3}\,M^{-1}\,s^{-1}$)
PPP	4.32	0.86	0.86
PPC	4.05	1.25	1.25
LMM	5.02	0.95	0.95
EPH	4.25	0.97	0.97
TPM	4.75	0.90	0.90

collisional quenching constant, K_q was then obtained from the slope of $[F_o/F\exp(V[Q])\}] - 1$ versus $[Q]$ plots through origin. The value of V and K_q so obtained [21] are recorded in Table 3. The value of V is found to be close to $5 \times 10^3\,M^{-1}$ in most cases.

According to Eftink and Ghiron [22] upward curvature in the Stern–Volmer plot indicates that both tryptophan residues of BSA are exposed to quencher and the quenching constant of each tryptophan residue is nearly identical, while downward curvature indicates buried tryptophan residues. At a concentration of 120 μM drugs, about 80% of the fluorescence intensity was quenched. The maximum quenching was obtained by extrapolating a plot of $(F_o - F)/F_o$ versus $1/[Q]$ to $1/[Q] = 0$ corresponding to infinite concentration of drug. We have observed that at infinite concentration of drug, fluorescence quenching was more than 90% in each case. This again shows that both the tryptophan residues of BSA are accessible to drug molecules. For a bimolecular quenching process, $K_q = k_q \times \pi_o$, where π_o is the lifetime in the absence of quencher and k_q is the rate constant for quenching. As π_o value for tryptophan fluorescence in proteins is known to be $=10^{-9}$ s [2], the rate constant, k_q, would be of the order of $10^{13}\,M^{-1}\,s^{-1}$. The value of k_q depends on the probability of a collision between fluorophore and quencher. This probability depends on their rate of diffusion (D), their size and concentration. It can be shown that

$$k_q = 4\pi a D N_a \times 10^{-3} \quad (5)$$

where D is the sum of the diffusion coefficients of quencher and fluorophore, a the sum of molecular radii and N_a is the Avogadro's number. The upper limit of k_q expected for a diffusion-controlled bimolecular process is $10^{10}\,M^{-1}\,s^{-1}$. The high magnitude of k_q in the present study ($10^{13}\,M^{-1}\,s^{-1}$) can probably be attributed to increase in the encounter radii of tryptophan–phenothiazine derivatives. This can happen only by the process of energy transfer and hence the quenching of tryptophan fluorescence occurs by intermolecular interaction forces.

3.3. Thermodynamics of drug–protein interactions

Thermodynamic parameters for the binding of drugs to BSA were determined Other conditions being same as described previously in experimental section. The association constant, K was found to decrease with increase in temperature since

$$\log K = -\frac{\Delta H_o}{2.30RT} + \frac{\Delta S_o}{2.30R} \quad (6)$$

where $\log K$ versus $1/T$ plot enabled determination of standard enthalpy change, ΔH_o and standard entropy change, ΔS_o for the binding process. The ΔH_o, ΔS_o and ΔG_o values found to be in the range of +72.7 to $+139.0\,kJ\,mol^{-1}$, +80.80 to $+560.05\,J\,K^{-1}\,mol^{-1}$, and −22.56 to $-29.9\,kJ\,mol^{-1}$, respectively. The positive values found for the enthalpy and entropy changes seem to indicate that the hydrophobic contribution is the predominant intermolecular force stabilizing the BSA-phenothiazine complexes. These results together with spectral changes in the fluorescence emission spectra of BSA induced by phenothiazine derivatives, suggest that the interaction may take place in subdomain I and II since these subdomains have been proposed to bind drugs and other hydrophobic materials [23].

3.4. Binding studies in the presence of ANS

Fluorescence spectra of 10 μM BSA in the presence of increasing amount of drug (4–25 μM) and ANS (5 μM) were determined upon excitation at 296 nm. Both drug and ANS quench the fluorescence of BSA,

but the magnitude of decrease in fluorescence intensity was much larger for ANS as compared to that for drug. The ANS bound to BSA calculated from the fraction of occupied sites (θ) was 76% where as the drug bound to BSA was around 23% under identical conditions. It is known that excitation at 296 nm involves fluorescence due only to tryptophan residues of BSA. Further, under conditions of the experiment, tryptophan residue of BSA are partially exposed and their accessibility depends upon the nature of molecules of the interacting species [24]. It thus appears that whereas, tryptophan residues are fully accessible to the hydrophobic probe, ANS they are only partially accessible to the drug which has partially hydrophilic character. Thus, drug and ANS do not share common binding site in BSA.

In another set of experiments, BSA–ANS interaction was studied in the presence and absence of 5, 10, 15, and 20 μM of each drug by monitoring ANS fluorescence upon excitation at 370 nm. It was found that for a given concentration of ANS, fluorescence intensity increases when drug is added to BSA–ANS system. It is known [25] that the ANS shows greatly increased fluorescence as a result of hydrophobic interactions with proteins and other macromolecules due to transfer of probe from an aqueous to non-polar environment. Increase in fluorescence intensity of BSA–ANS system on the addition of drug can be explained as follows: when drug is added to BSA–ANS system, it can compete with ANS for the hydrophobic sites on the surface. In that case it would inhibit the binding of ANS, i.e. displace ANS from its binding sites and the fluorescence intensity should decrease. But, the fluorescence intensity actually increases. This shows that ANS and drug do not share common sites in BSA. Increase in fluorescence intensity shows that the drug has the highly hydrophobic character, and thus, further shifts the fluorescence intensity of ANS to higher values.

To understand further the nature of interaction involved, CMC of an anionic surfactant, SDS was determined in the presence and absence of 25 μM drugs. The ANS is virtually non-fluorescent in aqueous solution and becomes highly fluorescent in non-polar solvent. Large increase in the intensity of ANS fluorescence on association of surfactant monomers to form micelles was employed as the basis of CMC determination. Fluorescence intensity of ANS (120 μM) was determined in the presence of increasing concentration (2.0–10 mM) of SDS upon excitation at 370 nm. Relative fluorescence intensity at λ_{max} (482–486 nm) was plotted against the concentration of SDS. It was found that presence of drugs decreased the CMC of SDS to 5.9×10^{-4} mM. The decrease in CMC shows that the positively charged *N*–moiety of branched chain of drug molecules interact with negatively charged anionic head groups at the boundary of SDS micelle thereby decreasing the CMC. The small decrease in CMC value in the presence of drugs shows that the drugs also have some ionic character in addition to the predominantly hydrophobic character.

3.5. Surface activity

Surface tension data was expressed as surface activity, which in turn expressed as surface pressure, π, which is the difference between surface tension of the solvent and that of the solution. Thus, surface activity values, expressed as surface pressure, $\pi = \lambda_{solvent} - \lambda_{solution}$, were found to be 2.5×10^{-2}, 4.64×10^{-3}, 8.34×10^{-3}, 1.8×10^{-2} and 3.04×10^{-3} for TPM, PPP, PPC, LMM and EPH, respectively. Reduction in surface tension of solvent or increase in surface pressure is attributed to hydrophobicity of the drug molecule. However, the order of π value suggests that the drugs have hydrophobic character.

3.6. Effects of additives and paracetamol

To understand further the nature of interaction involved, fluorescence spectra of albumin-phenothiazines were recorded in the presence of each of 20 μM urea, dextrose, magnesium stearate and starch, and saturated lauric acid solution. The increase in fluorescence intensity of albumin–drug mixture in the presence of urea, dextrose and starch shows that they weakened the hydrophobic interactions thereby inhibiting drug-BSA interactions while, the decrease in fluorescence intensity values in case of magnesium–stearate and lauric acid indicate that they facilitates hydrophobic interactions because of their lengthy and non-polar nature. Thus, urea, dextrose, magnesium–stearate, starch and lauric acid alter the microenvironment of the binding sites by affecting the iceberg structure of water. It may thus be concluded that hydrophobic interaction plays a significant role in the

Table 4
Relative fluorescence intensity of BSA-drug system in the presence of additives for phenothiazines

Sample	Fluorescence intensity at 344 nm				
	TPM	LMM	EPH	PPP	PPC
Only BSA	100	100	100	100	100
BSA + drug	89.21	94.79	81.18	82.97	72.46
BSA + drug + urea	92.18	95.74	83.59	85.24	76.78
BSA + drug + dextrose	92.14	99.01	84.22	83.68	75.67
BSA + drug + starch	92.43	98.49	81.46	87.00	79.40
BSA + drug + magnesium stearate	88.36	94.19	81.03	79.70	66.23
BSA + drug + lauric acid	83.72	91.18	78.11	79.58	68.45

interaction of phenothiazine derivatives to serum albumin. The results of analysis are given in the Table 4.

The simultaneous administration of two or more strongly bound drugs can compete with one another for the binding sites on albumin and so result in displacement interactions [26,27]. Although paracetamol is not strongly bound at therapeutic concentrations, it can still affect the protein binding behavior of other drugs either by blocking an active site or by causing conformational changes in the protein molecule. Thus, the presence of paracetamol can significantly alter the pharmacological response of other drugs by altering the concentration of free drug in plasma.

It was observed that the association constants of all phenothiazines almost decreased in the presence of paracetamol. In other words, the availability of free drug in plasma gets increased in the presence of paracetamol (Table 5). Once the interference of the paracetamol in the protein binding of drug is established one can anticipate the need for an adjustment in dosage in the presence of paracetamol. The relative ability of paracetamol to interfere in the binding of other drugs can be quantitatively determined from K_{ratio}, the ratio of association constant in the presence and absence of paracetamol. The K_{ratio} can, therefore, be a guide to the modified design of dosage forms in the presence of paracetamol.

3.7. Circular dichroism studies

The binding of phenothiazines was also confirmed by CD spectra. Typical CD spectra of a representative member of phenothiazines, PPC with BSA are shown in Fig. 3. As expected, the α-helices of protein show a strong double minimum at 220 and 209 nm [28]. The intensities of this double minimum reflect the amount of helicity of BSA and indicate that BSA contains more than 50% of α-helical structure. Upon addition of the drug to BSA (1:1), the extent of α-helicity of the protein decreases and hence the intensity of double minimum is reduced. On increased addition of drug to BSA (3:1), the double minimum start vanishing and later at 5:1, the intensity of the double minimum increased. This is indicative of increase in helicity when the drug is completely bound to BSA.

3.8. Equilibrium dialysis method

The effect of concentrations of drugs [TPM, LMM, EPH, PPP or PPC] on the binding to BSA was studied

Table 5
Association constants and percentage of drug bound of various drugs in the absence and presence of paracetamol

Drug	Association constant (K) ($\times 10^{-4}$ M^{-1})		Percentage of drug bound		K_{ratio}
	In presence of paracetamol	In absence of paracetamol	In presence of paracetamol	In absence of paracetamol	
PPP	4.55	7.61	11.85	14.27	0.60
TPM	4.32	6.21	12.68	23.68	0.69
LMM	3.96	5.55	14.68	21.68	0.71
PPC	3.68	4.85	17.69	19.77	0.76
EPH	3.45	4.32	18.75	20.05	1.0

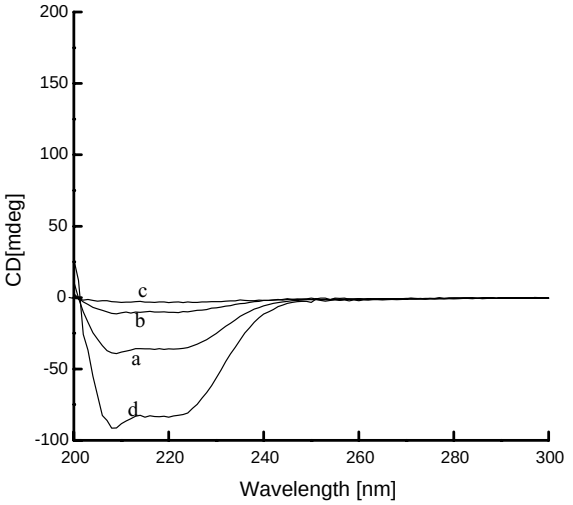


Fig. 3. Circular dichroism spectra in the 200–50 nm range; (a) BSA, 0.1 μM; [BSA]:PPC = 1:1 (b), 1:3 (c) and 1:5 (d).

by dialysis experiment. In these studies the concentrations of drugs were varied in the range of 1.0–6.0 × 10^{-5} M and keeping the protein concentration constant (1.0%). Examination of data has revealed that the binding increases with increasing concentration of phenothiazine at low drug–protein ratios. The amount of free drug remains the same in spite of fact that concentration of phenothiazine was further increased suggesting that higher number of binding sites on BSA, a similar observation made in the case of binding of phenoxazines to BSA [29]. In order to evaluate the association constant, *K* and the number of binding sites, *n*, the Scatchard plot was recorded. It was observed that the *K* and *n* values were within the error of ±5.0% to those obtained by spectrofluorometric method. Comparison of *K* values within the drugs examined also showed that the phenothiazine drugs bind to BSA in the order: TPM > LMM > EPH > PPP > PPC. This order is in agreement with that observed in spectrofluorometric method.

4. Conclusion

This work is an example of rarely encountered study wherein the interaction of BSA with selected drugs has been investigated by employing fluorimetry, circular dichroism and equillibrium dialysis methods. The obtained results suggest that the phenothiazines bind to BSA possibly by hydrophobic interactions. The shape and intensity of negative CD bands at 209 and 220 nm show major differences in the presence of phenothiazine drug due to change in the chemical environment of α-helices lying at the surface of the protein. Complete binding of phenothiazine drug to BSA is found to induce conformational changes in protein leading to increase in the helicity of protein.

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